Abstracts

Applying New Biotechnology to the Study of Occupational Cancer

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CHALLENGE OF APPLYING NEW BIOTECHNOLOGIES TO THE STUDY OF OCCUPATIONAL CANCER: A PRESPECTIVE FROM ACADEMIA

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There currently is a vast armamentarium of biomarkers available for monitoring workers potentially exposed to carcinogens in the workplace. Added to these are the high throughput molecular methods that are continually emerging from the areas of toxicogenetics, toxicogenomics and proteomics for assessing biological responses to hazardous agents. Despite this, several challenges remain for studies of occupational cancer. First, although there is an abundance of biomarkers in the traditional categories (i.e. exposure, effect and susceptibility), there are some deficiencies. For example, there are few measures of carcinogen exposures that occurred in the distant past. Beyond this however, there is also a need for biomarkers in new categories, i.e. a need for measures of *in vivo* processes underlying cancer that are not encompassed by the traditional categories. Such biomarkers are almost totally lacking. "Clonality" and "selection" are examples of two *in vivo* processes for which the new biotechnologies could be called upon to develop usable indicators. Second, even the most sophisticated of molecular analysis will be limited by the biological materials available. Obtaining the appropriate materials from humans, especially cells, is a major challenge. Third, all biomarkers used in human studies must be thoroughly validated as to use. Validation for each kind of biomarker will pose its own challenges. Fourth, studies of workers are best designed using a continuum of biomarkers to provide for internal validation. Although expensive, this is especially important for interpreting negative effects in the context of potentially hazardous external exposures. Finally, to the extent possible, human population studies and animal experimental studies should have parallel designs so that results can be compared. Although this may change some of the ways each is traditionally conducted, human-animal comparisons will be particularly valuable for exploring low dose effects in humans, for indicating the most relevant biomarkers for human monitoring in given exposure situations and for providing a mechanistic basis for risk assessment. Examples from studies of 1,3-butadiene will be used to illustrate the fourth and final points in this presentation.

DNA DAMAGE AND SOMATIC MUTATIONS FOLLOWING EXPOSURE TO OCCUPATIONAL CARCINOGENS

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Laboratory investigations have demonstrated the interaction of occupational carcinogens with DNA and the induction of genetic alterations in the form of DNA adducts, cytogenetic damage and mutations in specific reporter and cancer genes. Using similar biological endpoints, these observations have been extended in molecular epidemiological studies of exposed subjects including the demonstration of mutational fingerprints characteristic of specific exposures in cancer genes in human tumors. These experimental approaches provide complementary biological information. Methods measuring levels of DNA adduction offer excellent experimental sensitivity and specificity for individual carcinogens but the relationship between the level of measured adducts and biological implications are uncertain; *in vitro* studies of specific locus somatic mutation provide greater biological relevance since these early biological effect markers lie directly along the mechanistic pathway between exposure and carcinogenesis. Finally, molecular epidemiological studies in occupationally exposed populations provide biomarker data that integrate the genotoxic impact of cumulative exposure to complex mixtures theoretically in the context of directly measurable health outcomes, i.e., cancer incidence. However, these methods presently lack both sensitivity and specificity for individual occupational genotoxic agents and such large prospective population-based studies are both challenging to establish and expensive to carry out.

In this presentation, I will summarize the present literature with illustrations of these approaches with an emphasis on the application of *in vivo* specific locus somatic mutation assays in small-scale occupational cohorts exposed to benzene, butadiene, coke oven emissions, ionizing radiation, and styrene. These examples will serve to illustrate both the strengths and weaknesses of the present assay systems and epidemiological approaches. Lastly, I will speculate on the need for the development of more robust and relevant mutational assay systems that could potentially be developed using newly emerging genomic information and biotechnological approaches.

OCCUPATIONAL CANCER IN THE 21ST CENTURY: WHAT ARE THE GREATEST CHALLENGES?

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Occupational investigations have made a major contribution to our understanding of environmental causes of cancer. Over half of the substances labeled as human carcinogens by IARC were discovered in the occupational arena. Despite this long and successful contribution, there is a perception among scientists and the public that studies of occupational exposures are unlikely to significantly expand our knowledge of cancer etiology in the future. This conclusion follows from premises that assume nearly all carcinogens in the workplace have been identified, exposures still occurring are well under control, and workplace exposures cannot provide useful information in the genome era. These assumptions are faulty. There are a large number of occupations that experience elevated rates for selected cancers for which the specific hazard involved has not been identified, e.g., agricultural workers, bakers, firefighters, meat workers, painters, and foundry workers. In addition, experimental and epidemiologic studies have identified a number of workplace exposures that may be carcinogenic, but which require further investigation, e.g., pesticides and solvents. Exposures may be well controlled in large plants in developed countries, but they are less so in small businesses and in the developing world. Although previous studies of workplace exposures have made major contributions to our understanding of carcinogens and the carcinogenic process, they have been largely based on populations composed of white men in developed countries. We should not base our understanding of occupational carcinogenesis so narrowly. In the era of the genome, there is great excitement and optimize that the carcinogenic process can be elucidated. Interdisciplinary investigations that simultaneously consider exposures, genetic factors, and intermediate markers will significantly expand our understanding of the carcinogenic process. There is no better place to do this than in the occupational arena which has relatively high levels of exposure (at least compared to the general environment) and distinct and sometimes well-characterized exposure patterns. Such interdisciplinary approaches will require development and validation of intermediate biologic markers, careful consideration to design issues to insure reliable and valid collection of biologic tissue, and special attention to the vexing ethical issues associated with biologic research in epidemiology.

VALIDATION AND LINKING INTERMEDIATE BIOMARKERS TO CANCER

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The validation of biomarkers as early predictors of clinical disease is a leading priority in the field of occupational medicine and environmental research. The availability of validated biomarkers can enhance health risk assessment and contribute to effective new disease prevention policies. In the validation process the relationship between the frequency of a biomarker and the incidence of disease is generally established by longitudinal studies. These studies are costly and time consuming but involve subjects that are healthy at the time of testing, avoiding in this way the reverse causality bias, i.e., the possibility that the biomarker is affected by the disease or its treatment. A few biomarkers, sometimes defined as *Cancer Risk Biomarkers*, have been validated through longitudinal studies; among these DNA adducts and especially chromosomal aberrations in blood lymphocytes are those with the most circumstantiated evidence. The information provided by the distribution of these biomarkers in human populations can be exploited for an early identification of groups at risk and susceptible individuals. The possible application of chromosomal aberrations as a screening test will be discussed in three examples referring to population groups with known or highly suspected risk of cancer, i.e., radiation workers, astronauts and alcoholics. These groups are largely heterogeneous as regards the number of exposed individuals, the extent of risk and the social context, but share the common difficulty of identifying effective cancer control policies. The recent accumulation of evidence supporting the link between some intermediate endpoints of cancer and the clinical outcome encourages the use of biomarkers of risk in *precautionary prevention* programs of occupational cancer.

Poster

THE SHANGHAI WOMEN'S HEALTH STUDY: A PROSPECTIVE COHORT OF 75,000 WOMEN FOR STUDY OF OCCUPATIONAL AND GENETIC CANCER RISKS

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Cancer incidence rates vary substantially among countries. Epidemiologic studies provide opportunities to identify environmental and susceptibility factors that may explain international variation in disease rates and provide new insight into cancer etiology. During October 1997 and April 2000, the Shanghai Women's Health Study recruited 75,049 women aged 40-70 years residing in seven urban communities of Shanghai, China, yielding a response rate of 93%. A spot urine was collected from 88% of the participants, and 10 ml of blood from 76%. For those who did not provide blood, a buccal cell sample was collected, yielding a DNA sample for over 88% of the cohort. These response rates are among the highest for cohort studies worldwide, allowing for estimation of population-attributable risks. Blood, buccal cell, and tumor tissue slides will be collected from newly diagnosed cases. Follow-up for cancer incidence and mortality will be achieved by biennial re-contact with the subjects and linkage to a population-based tumor registry and a vital statistics registry. By 2007, 3,935 incident cancer cases are expected, including 3,506 with DNA samples. Noteworthy characteristics of this cohort include low consumption of alcohol (2%) and tobacco (3%), low intake of dietary calorie from fat (15%), high intake of soyfoods, and near 100% employment with 60% in blue collar jobs. Occupational exposure assessment is enhanced by an ongoing validation study and an exposure database based on industrial monitoring by the Shanghai municipal government.

USE OF GENOMICS IN TOXICOLOGY AND EPIDEMIOLOGY

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The advent of new biotechnologies has created exciting possibilities for application to worker health and safety issues. Many of these technologies have the potential to significantly advance our ability to explore and address issues in toxicology, epidemiology, and risk assessment. While the science of 'omics' is advancing rapidly, the requisite complementary science is lagging for scientific agreement on the meaning of results and for a thorough understanding of consistency and variability in samples and study designs. Much of the research energy has been directed to advancing the tools; little has been used to correlate the 'omics' findings quantitatively or qualitatively to exposure, dose, or adverse effect. This apparent disconnect also reflects the lack of a critical mass of data and bioinformatic tools.

The chemical industry is committed to acquiring the knowledge to protect the environment and the health of people and wildlife, and the science of 'omics' holds great promise to advance this knowledge. Therefore, the American Chemistry Council is using these technologies in research it supports at the CIIT Centers for Health Research and in joint programs with the U.S. Environmental Protection Agency (EPA), and the National Institute of Environmental Health Sciences (NIEHS).

Four themes for improving the use of genomics have been recommended: *1) "Omics" technology should be used in a framework of toxicology and epidemiology principles so that it can be related and applied in a context that is understood for risk assessment; 2) effective application of "omics" to epidemiology studies will require suitable biological samples from large and diverse population groups at relevant times of exposure; 3) ethical, social, and legal perspectives will require involvement of non-scientific stakeholder communities; and 4) a unified research agenda as applied to toxicology and epidemiology is urgently needed in order to realize the potential power and benefits of these new technologies.

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ASSESSING CHROMOSOMAL ALTERATIONS IN EXPOSED HUMAN POPULATIONS

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Structural and numerical aberrations are observed at increased frequencies in the lymphocytes of workers exposed to a variety of carcinogenic agents. A growing body of molecular and cytogenetic evidence indicates that similar genetic alterations play a critical role in neoplastic development. Consistent with this, individuals with elevated frequencies of structural aberrations in their lymphocytes have been shown to be at an increased risk of developing cancer. The association between aberrations and cancer has stimulated the development of new techniques to enhance the detection of chromosomal alterations in chemically exposed populations. In this presentation, I will overview a number of the strategies for detecting chromosome-level changes in human cells, and briefly discuss their advantages and limitations for use as biomarkers. Illustrations will include more established techniques such as metaphase analysis of unbanded chromosomes, the CREST-modified micronucleus assay, and fluorescence in situ hybridization for the detection of aneuploidy and structural aberrations, as well as more recent developments such as spectral karyotyping and rolling circle amplification.

Poster

SELECTION AND DEVELOPMENT OF BIOMARKERS FOR CANCER DETECTION AND RISK ASSESSMENT

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There is a need for improved methods for detecting individuals at risk for cancer to target subsets of patients for more intensive individual screening, and targeted chemoprevention. One approach for accomplishing this objective is to detect premalignant molecular fingerprints in an organ at risk for cancer. Bladder cancer is an excellent model for testing this approach; however, comprehending the strategy for biomarker selection and analysis is more complicated than is generally appreciated. The objective of this poster is to provide a succinct overview of our experience with the selection of biomakers for bladder cancer detection, first in symptomatic patients and then in a high-risk cohorts of workers at risk for bladder cancer. Biomarker selection depends on multiple parameters, each of which must be optimized to enhance the utility of a biomarker for clinical application. Many markers that initially show promise fail in the clinical arena for a variety of reasons. Important parameters include, when a biomarker is expressed in carcinogenesis (i.e. early vs. late), the sample type, method of analysis, all contribute to the sensitivity, specificity, and ultimate clinical utility of a biomarker(s). Virchow has suggested all diseases start in the cell and Seymore West indicated the cell under appropriate conditions can function as a microcuvette for biophysical cytochemical analysis. Spectroscopy provides an accurate and sensitive method for quantitative single cell proteomics. Improved and more stable fluorescence probes and a rationale approach for biomarker selection based on the concepts of field cancerization, complemented by improved quantitative analysis of protein markers at the single cell level will enhance the utility of cellular chemistry; a platform for single cell proteomic analysis that can be applied to multiple basic science and clinical problems. Single cell proteomics also facilitates the study of genetic instability, epigenetic signaling (stromal-epithelial interactions) in relation to cancer therapy and diagnosis. Because most cancers arise through multiple signaling pathways, and are heterogeneous, the identification of appropriate biomarker profiles provides a number of strategic advantages over a single biomarker. Complex networks of signaling pathways lead to increased cell proliferation, decreased cell adhesion, cellular differentiation, genetic instability, and other functions associated with the malignant phenotype. Summarized here are the fundamental concepts for biomarker(s) selection and profile analysis of high level phenotypic biomarkers developed for bladder cancer risk assessment, screening, and early bladder cancer detection.

IDENTIFICATION OF RELEVANT GENES FOR KNOWN OR SUSPECTED HUMAN CARCINOGENS

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Known or suspected occupational carcinogens include aromatic amines (AAs), polycyclic aromatic hydrocarbons (PAHs), nitrosamines, mycotoxins, as well as certain simple monoaromatic, vinyl, and alkenyl compounds. Although there are over 30 human cytochromes P450 (CYP), only CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2C9, CYP2E1, and CYP3A4 play a major role in the metabolic activation of these carcinogen to proximate or ultimate carcinogens. Each of these genes or their enzymes exhibits a polymorphic that results in differences in individual susceptibility in molecular epidemiologic studies. In addition, epoxide hydrolase (HYL1) polymorphisms are important in cancer risk thought to be attributable to PAHs, as epoxides must be hydrolyzed in order to form the DNA-reactive metabolites. Similarly, for AAs, acetyltransferases (NAT1 and NAT2) and sulfotransferase (SULT1A1), which are all polymorphic, these can serve to detoxify (*N*-acetylation, *N*–sulfonylation) or activate (*O*-acetylation; *O*-sulfonylation), depending on the substrate. Phenotypic studies indicate that several of these genes have yet to be defined single nucleotide polymorphisms (SNPs). A useful approach for SNP discovery currently underway in our laboratory involving DHPLC will be described.

Poster

IDENTIFICATION OF PROTEIN BIOMARKERS IN MAGNETIC FIELD (MF)-TREATED HUMAN GLIOMA CELLS

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As part of an established role in protecting worker safety and health, the National Institute for Occupational Safety and Health (NIOSH) investigates the effects of suspected occupational carcinogens on cancer-linked molecular events in appropriate models. Here we investigate the effect of low level magnetic field exposure on phenotypic changes in SF767 human glioma cells, possible protein biomarkers. We study biochemical alterations of proteins after exposure to 1.2 microTesla (μT), [12 milliGauss (mG), 60 Hertz (Hz)] MF with or without (+/-) epidermal growth factor (EGF). SF767 cells are maintained in 10% fetal calf serum (FCS)-Dulbecco's Modified Eagle's Media (DMEM) until they reach 70% confluence. The cells are harvested and exposed for 3 hr to sham conditions (<0.2 μT ambient field strength) or 1.2 μT MF (+/- 10 ng/ml EGF). Following exposure two dimensional polyacrylamide gel electrophoresis (2D-PAGE) is used to resolve and characterize glioma cell proteins by molecular weight (Mr) and isoelectric point (pI). Solubilized protein fractions from each treatment group (sham; sham + EGF; 1.2 μT; 1.2 μT + EGF) are loaded for electrophoresis by 2D-PAGE and stained using a colloidal Coomassie blue technique. Using PDQUEST software, protein patterns across groups are compared via Student's t-test. Following computer analysis automated spot excision and processing is performed prior to peptide mass fingerprinting proteins of interest. Fifty-two proteins were identified by this technique, and a subset of these proteins were found to differ significantly across treatment groups. The mean abundance of ten identified proteins was altered following 1.2 μT exposure relative to sham (3 increase, 7 decrease). The identification and functional role of these protein biomarkers in human glioma following low level magnetic field may offer some early insight into the putative role of magnetic field exposure in human cancer risk.

Poster

EXPRESSION PROFILING OF PRIMARY NORMAL HUMAN MAMMARY EPITHELIAL CELLS IN RESPONSE TO BENZO[A]PYRENE EXPOSURE USING OLIGONUCLEOTIDE MICROARRAYS

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Molecular epidemiological studies have implicated certain common polymorphisms with risk of breast cancer, and have suggested that gene-gene or gene-environment interactions may be important. Inter-individual variations in carcinogen metabolism together with differences in DNA-repair capacity potentially govern the relative risk of an individual to chemical exposures. The goal of this study, consistent with the NIOSH mission, is the development of biomarkers of occupational exposures. Therefore, we have explored altered gene expression patterns in a panel of four primary normal human mammary epithelial cell strains developed from normal healthy breast tissue obtained at reduction mammoplasty through the Cooperative Human Tissue Network (NCI and the NDRI, sponsors). The cell strains were exposed to benzo[a]pyrene (4 μM) for 0, 2, 12, 18 and 36h. Gene expression was monitored in cells harvested at these time points using high-density oligonucleotide arrays (Affymetrix HuGeneFL). Total RNA was used for the preparation of labeled targets and hybridized to microarrays containing probes representing more than 6800 full-length human genes and expressed sequence tags. Gene expression data for unexposed and benzo[a]pyrene exposed cell strains were analyzed using Affymetrix GeneChip™ software. To demonstrate consistency of cRNA synthesis and transcript labeling between different samples over the duration of exposure, the cRNA 3'/5' transcript ratios were compared between treatment groups for GAPDH and β-actin. The 3'/5' cRNA transcript ratios for both GAPDH and β-actin were found to be consistent between samples over the duration of exposure. All treatments were performed in duplicate and displayed a high degree of reproducibility. The data show altered gene expression patterns (≥100% change) in >500 RNA species in response to benzo[a]pyrene exposure. Specifically, it was observed that PCNA, a cell cycle control gene, and its promoter were over expressed in all the four cell strains. The dioxin inducible cytochrome P450 *CYP1B1*, involved in steroid metabolism and the metabolic activation of benzo[a]pyrene was also induced in all four cell strains. In addition, altered gene expression was found in other carcinogen metabolism genes, DNA repair genes, and cell cycle regulation genes. These data provide an overview for inter-individual susceptibility to chemical carcinogen exposure. This approach may ultimately be useful for analysis of gene-environment interactions and development of biomarkers of occupational exposures.

ROLE OF GENOMIC TECHNOLOGY IN OCCUPATIONAL CANCER PREVENTION

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Our environmental and occupational cancer prevention project rests on premises that: observed increased cancer in a population with high exposures to an agent predicts risk at much lower exposures; carcinogenicity in laboratory bioassays predicts risk in people; and, enough people are exposed to enough carcinogens to compel a major enterprise in exposure control. These rest in turn on the mechanistic paradigm of cancer arising from alteration of DNA, a cellular level effect which predicts cross species concordance and low dose continuity with no practical population threshold. Testing these paradigms is constrained by our "Limit of Direct Observation (LODO)" in a population of at least 10% tumors in a standard bioassay, and 1-10% attributable cancers in a mortality study. Experience with radiation, asbestos, cigarette smoke, AAF and nitrosamines provides no empirical evidence for a population threshold. Genomic technology shows promise for detection of carcinogenic effect in a population exposed below the LODO dose. The danger of genomic technology is providing raw materials for the Houdini Risk Assessment industry. Research should be directed at agents and exposure circumstances of occupational health importance known or probably carcinogenic to humans, rather than model compounds. Priorities include: particulate matter generally, silica, diesel particulate matter, metal working fluids, welding fume, sulfuric acid mist, solvent vapors, and formaldehyde.

INHERITED MODIFIERS OF RISK IN OCCUPATIONAL CANCER

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Ecogenetics can be defined as "the study of variability in the heritable response to all (chemical and physical) environmental agents." *Ecogenomics* might be defined as "the study of how the signal of any (chemical or physical) environmental agent interacts with the expression output of the entire genome (genetic architecture) to influence biological pathways and processes."

Between 5 and 30 years ago, it was believed that "environmental susceptibility genes" represented primarily those genes that encode drug-metabolizing enzymes (DMEs). As the Human Genome Project is beginning to tell us, however, ***all*** genes are highly polymorphic—with perhaps a several-fold range of variability on a gene-by-gene basis. This realization suggests that perhaps every gene in the human genome might be regarded as an environmental susceptibility gene. For the past five decades, most studies have concentrated on what the body does to environmental chemicals (*ecokinetics*)**;** in the past decade, as more knowledge has been gained about signaling pathways, studies have begun to focus on receptors, transporters, signal transduction pathways and transcription factors as we learn what each environmental signal might do to the body (*ecodynamics*). This includes the fact that environmental agents can act as either agonists or antagonists, or as activators or inhibitors, in every signaling pathway in the body. This also includes the realization that the genetic makeup of the individual can affect the results from biomarker monitoring in the work place. This explosion in knowledge about human genetics and genomics should help us in gaining a better understanding the mechanisms involved in occupational carcinogenesis, but also demonstrates how complex this field actually is. Moreover, this explosion in knowledge is creating an increasingly large gap between wet-bench science and ethical, legal and social issues surrounding the choices of who (*i.e.* anyone besides the individual worker?) should know the genotype of any particular worker.

USING BIOMARKERS IN ASSESSMENT OF EXPOSURE TO OCCUPATIONAL CARCINOGENS

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Levels of exposure to occupational carcinogens vary dramatically from day to day. Substantial variability, regarding the rates of uptake, elimination, and metabolism of the carcinogens, also exists between individuals. These sources of variability have motivated use of biomarkers to assess exposures to occupational carcinogens. Yet, due to differences in residence times of biomarkers, they are not all equally well suited for exposure assessment, dosimetry, and epidemiology. Intermediate- and long-term biomarkers, such as, hemoglobin and albumin adducts, heavy metals in blood or urine, and lipophilic organic compounds in blood or fat, damp exposure variability from day to day and, thus, are useful for these purposes. However, short-term biomarkers, such as small organic molecules in blood or breath, urinary metabolites, and lymphocytic DNA adducts, are generally no more useful than environmental measurements for these purposes. But regardless of their residence times, all biomarkers are extremely useful for evaluating human kinetic processes, *when used in conjunction with environmental measurements in longitudinal studies*. These points will be illustrated with biomarkers of benzene in some occupational studies.

HOW TO ASSESS POSSIBLE HEALTH EFFECTS OF SOLVENT EXPOSURE

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Background: Epidemiological studies consistently show increased risk for cancer among women dry-cleaning workers. Over 90% of an estimated 50,000 U.S. drycleaning shops currently use tetrachloroethylene (perchloroethylene, PCE) as their primary dry cleaning solvent. PCE is a recognized animal carcinogen classified by the International Agency for Research on Cancer (IARC) as a probable human carcinogen. Increased risk for some cancers in dry cleaners has been attributed to life-style or medical access risk factors, or possibly to other solvents, in previous epidemiological studies of cervical cancer in solvent-exposed workers.

Problem: How can we ascertain whether there is a connection between exposure to PCE and health effects, such as cancer?

Methods: This question was investigated in a NIOSH pilot project designed to explore the feasibility of and refine the methods to be used in a full-scale study. The experimental design is presented here. In the pilot project 18 women working in dry cleaning were compared with 20 women working in industrial laundries, matched by age, race, and smoking status. Other investigators, especially a group at the University of Wurzberg, Germany, are studying PCE metabolism.

Results: The NIOSH pilot study succeeded in collecting environmental samples and biological specimens and in processing and distributing samples and specimens to laboratories. We collected 97% of scheduled blood specimens, 95% of gynecological specimens, 100% of four core urine specimens requested from each participant and 86% of urine specimens requested from exposed participants to analyze variability over a three-week exposure period, and sent aliquots to 15 laboratories across the United States. Over thirty biomarkers of exposure, effect, and susceptibility were analyzed. Some analyses have been completed; others are on-going.

Conclusion: It was not expected with a group this size to find significant health status differences between dry-cleaning and laundry workers. However, there have been some results suggestive of an effect of PCE on health. These results, as well as the findings of multiple lifestyle risk factors, warrant a full-scale study, for which changes in study design have been suggested. A similar study design could be used to investigate a group exposed to another solvent.

APPLICATION OF NEW BIOTECHNOLOGIES TO THE UNDERSTANDING AND CONTROL OF KNOWN AND SUSPECTED OCCUPATIONAL CARCINOGENS

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This meeting is an effort to take stock of what new biotechnologies—particularly genomics, transcriptomics, and proteomics—can provide for occupational cancer research. The products of these new biotechnologies may be considered as biological markers, which over the last 25 years have been used and discussed in occupational and environmental health as part of a continuum of events between exposure and disease. The products of high-throughput and high-output technologies differ from classic biomarkers in the level of detail they represent. A large number of gene variants, transcripts, or proteins can now be assessed in a very short time. These products depict an increased level of complexity because they represent a more detailed and holistic data set. It is a bit naive to think that a single metabolic polymorphism is a major component in occupational cancer risks when many mechanisms acting in concert or conflict need to be considered simultaneously, as is possible with these technologies. However, before their utility is assured in occupational cancer research, a number of technical and scientific issues need attention. These issues include standardization of techniques, decisions about scales and outliers, and methods for comparing platforms and understanding the meaning of various perturbations of array patterns in relation to exposure, effect modification, or disease. If these technologies and their products can be validated in these regards, then the question becomes to what extent will they contribute to occupational cancer research?

A team of scientists from various sectors (The NORA Cancer Research Methods Team) has identified needs and gaps concerning research methods. Four areas were identified, and the challenge of this meeting is to see how new technologies can contribute to addressing these four areas: carcinogen identification, epidemiologic research, improvements in risk assessment, and prevention of occupational cancer.

Carcinogen Identification

Can the new technologies supplement or improve on ways of predicting and testing chemicals? Can the new technologies help in assessing mixtures?

APPLICATION OF NEW BIOTECHNOLOGIES TO THE UNDERSTANDING AND CONTROL OF KNOWN AND SUSPECTED OCCUPATIONAL CARCINOGENS (continued)

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Epidemiologic research

Can new biotechnologies improve historic exposure assessment, provide surrogate endpoints for disease, or enhance assessment of effect modification and confounding?

Can they provide new tools to understand the etiology of occupational cancer?

Improvements in risk assessment

Can new biotechnologies improve risk assessment by reducing uncertainty and modifying default assumptions for enriching biological models?

Can individual risk assessments be conducted using these technologies that determine whether a worker has been exposed or what is the cause of a particular cancer?

Prevention of occupational cancer

Can new biotechnologies create tools that will provide early warnings of carcinogen exposure or risk, demonstrate the effectiveness of interventions, or provide the basis for preventive recommendations?

Can new technologies be used to screen asymptomatic workers at increased risk of occupational cancer?

Plans are needed for concerted action to address each of these questions. New biotechnologies promise to contribute substantially to occupational cancer research. Our challenge is to apply them.

Poster

USE OF HIGH OUTPUT TECHNOLOGIES IN MOLECULAR EPIDEMIOLOGIC RESEARCH

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Cancer epidemiologists soon may have the opportunity to use high output technologies in research and prevention programs. These technologies, such as DNA microarrays, have the capability of providing a relatively quick analysis of sequence or expression for many thousands of genes simultaneously. How will these data be used and analyzed? To help answer those questions we looked at how epidemiologists have handled other situations which have multiple markers in a single study or analysis. We have reviewed issues that have surfaced when using four types of data: 1) batteries of complementary markers (such as DNA and protein adducts, cytogenetic effects and polymorphisms in genes involved in the metabolism of xenobiotics); 2) human leukocyte antigen (HLA) haplotypes; 3) mutational spectra; 4) protein sequence data from two-dimensional polyacrylamide electrophoresis (2D-PAGE). Additionally, we address future uses of high output technologies to assess exposure, control hidden confounding, enhance risk assessments, identify genetic variation in complex disease phenotypes, and reduce misclassification of disease. Prior to application in research and risk assessment microarray markers will need to be validated for these specific uses. Initially, the major challenge in the use of high output technologies will be to develop efficient ways to summarize the data. Eventually, the use of these technologies may facilitate the identification of potential hazards, may provide better biological dose information, and may serve as better benchmarks for the extrapolation of effects across species or exposure scenarios.

THE PROMISE OF NEW BIOTECHNOLOGIES FOR ASSESSING OCCUPATIONAL CARCINOGENS

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New technology offers great potential for advances in cancer biomarker research. I will describe a number of new biotechnologies and discuss their potential for use in occupational cancer epidemiology. The successful sequencing of the human genome has revealed several new insights, including the fact that human genome consists of more than 35,000 genes and is highly variable, with approximately 60,000 functional polymorphisms. High throughput genotyping of functional polymorphisms in key genes offers great potential for identifying individuals with susceptibility to occupational cancer but has problematic social and ethical implications. It is also now possible to observe the expression of all known genes using cDNA microarrays opening the possibility of new biomarkers of exposure and early effect. A current major area of biomarker research is proteomics, the study of the complete protein complement. It is possible with this methodology to examine novel proteins associated with disease or exposure in small samples of serum and cells. Future technologies will be based on nanotechnology and will provide new platforms for high throughput, highly sensitive, functional assays on a miniscule scale. All these new technologies will, however, require extensive validation in epidemiological studies.

EXPRESSION ASSAYS AND OVERALL CONCEPTS IN TOXICOGENOMICS

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The problems of identifying environmental factors involved in the induction and evolution of human disease, and of conducting safety and risk assessments of drugs and chemicals, have long been formidable issues. Three principal components for predicting potential human health risks are first, the diverse structure and properties of thousands of chemicals and other stressors in the environment; second, the time and dose parameters that underpin the relationship between exposure and disease; and third, the genetic diversity of organisms used as surrogates to determine adverse chemical effects. The techniques evolving from the successful genomics efforts are providing new exciting tools with which to address these intractable problems of environmental health and toxicology. In order to exploit the scientific opportunities, the National Institute of Environmental Health Sciences has created the National Center for Toxicogenomics (NCT). The primary mission of the NCT is to use gene expression technology, proteomics and metabolite profiling to create a reference knowledge base that will allow scientists to understand mechanisms of toxicity and to be able to predict the potential toxicity of new chemicals and drugs. Two important scientific issues in microarray analysis of chemical exposures are signature profiling of the action of drugs or chemicals, and microarray methodologies to determine biomarkers of exposure and potential adverse effects. The initial approach of the NCT is to carry out proof-of-principle experiments in an effort to "phenotypically anchor" the altered patterns of gene expression to conventional parameters of toxicity and to define dose and time relationships in which the expression of such signature genes may precede the development of overt toxicity. The microarray approach is used in conjunction with proteomic techniques to identify specific proteins that may serve as signature biomarkers. A long-range goal of these efforts is to develop a reference relational database of chemical effects in biological systems (CEBS) that can be used to define common mechanisms of toxicity, chemical and drug actions, to define cellular pathways of injury and response, etc. In order to implement this strategy, the NCT is creating a consortium of research organizations and private sector companies to actively collaborative in populating the database with high quality primary data. The evolution to a knowledge base of toxicogenomics will be accomplished through establishing relational interfaces with other sources of information on the structure and activity of chemicals, such as that of the National Toxicology Program, and to public databases annotating gene/protein identity and function.

Poster

PHOSPHORYLATION OF CHK1 ENHANCES RADIOPROTECTIVE P53-DEPENDENT CELL CYCLE ARREST PATHWAYS.

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Since DNA damage-inducible cell cycle checkpoints are thought to protect cells from the mutational effects of ionizing radiation, a better understanding of the mechanistic functions of cell cycle regulatory proteins may reveal new molecular targets for radiation carcinogenesis. Also, cell cycle arrest disfunction may be a good biomarker for individual sensitivity to radiation-induced cancer. Arrest at G2 is an important protective radioresponse for cycling cells. The two major regulatory proteins of G2 arrest are Chk1 and p53. Yet, it is unclear how these two proteins interact and coordinate their functional roles during radiation-induced G2 arrest. To determine Chk1's role in p53-dependent G2 arrest, we used p53 proficient cells and examined expression of G2 arrest proteins under conditions in which G2 arrest was inhibited by the staurosporine analog, UCN-01. We found that UCN-01 inhibited both G1 and G2 arrest in irradiated p53 proficient cells. The arrest inhibition was associated with suppression of radiation-induced expression of both p21 and 14-3-3σ -- two known p53-dependent G2 arrest proteins. The suppression occurred despite normal induction of p53, and normal phosphorylation of p53 at S20 and Cdc25C at S216 – the two known substrates of Chk1 kinase activity. In contrast, we showed that radiation-induced phosphorylation of Chk1 at S345 was associated with binding of Chk1 to p53, p21, and 14-3-3σ, and that UCN-01 inhibited S345 phosphorylation. We suggest that DNA damage-induced phosphorylation of Chk1 at S345, and subsequent p53 binding, links Chk1 with p53 downstream responses and may provide a coordinated interaction between DNA damage responses and cell cycle arrest functions. Disfunction or suppression of this pathway either through gene mutations or polymorphisms, respectively, may result in increased susceptibility to radiation-induced cancer.

METAL WORKING FLUIDS: CAN NEW TECHNOLOGIES HELP ASSESS CANCER RISK?

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Metal working fluids (MWFs) are in widespread use for the purpose of cooling and lubricating working surfaces during manufacture of metal products. Many millions of workers use MWFs, and the opportunities for dermal exposure or inhalation exposure, with concommitant ingestion, are substantial. The largest cohort study to date has reported excesses of esophageal, laryngeal, rectal, brain, prostate, liver and skin cancers among subgroups of exposed workers. As an exposure for epidemiologic study, MWFs present a number of methodologic challenges. Formulations are complex mixtures; a wide variety of formulations have been in use, some having no ingredients in common with others. With use, the fluids undergo chemical changes, and recycling and reprocessing of formulations compounds this issue. Many of the suspect component carcinogens (*e.g.*, polyaromatic hydrocarbons and nitrosamines) are unintentional contaminants, not listed in formulation descriptions. There have been secular changes in composition at ill-defined points in time such as changes in refining processes which led to a reduction in PAH content in the base oils used in some of the end products. Finally, there is coexposure to metals, abrasives, and other agents involved in metal working, making it difficult to distinguish among these agents with regard to their carcinogenic activity. Given the variability and complexity of the exposure, it is not surprising that there is a lack of consistency across epidemiologic studies of MWF-exposed populations. In this presentation, difficulties encountered in studying the carcinogenicity of this important occupational exposure using classical epidemiologic approaches will be summarized, setting the stage for a discussion of the possibilities offered by new biotechnologies.

Poster

OXIDATIVE DNA DAMAGE IN FEMALE DRY CLEANERS EXPOSED TO PERCHLOROETHYLENE (PERC)

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Oxidative DNA damage and lipid peroxidation were assessed in 38 women with (dry cleaners) or without (launderers) occupational exposure to Perchloroethylene (PERC). PERC exposure was assessed by collecting breathing zone samples on two consecutive days of a typical work week PERC levels were measured in blood drawn on the morning of the second day of breathing-zone sample collection in dry cleaners and before a typical workday in launderers. Significant correlations were noted between TWA PERC and blood PERC in dry cleaners. 8-Hydroxydeoxyguanosine (8-OHdG), ng/mg deoxyguanisine (dG) in leukocyte nuclear DNA was used as an index of steady-state oxidative DNA damage. Urinary 8-OHdG, μg/g creatinine was used as an index of oxidative-DNA-damage repair. Urinary 8-epi-prostaglandin $F_{2\alpha}$ (8-epi-PGF), ng /g creatinine was used as an index of lipid peroxidation. The mean $\pm$ SD leukocyte 8-OHdG in launderers was 16.0 $\pm$ 7.3 and was significantly greater than the 8.1 $\pm$ 3.6 value of dry cleaners. Urinary 8-OHdG and 8-epi-PGF were not significantly different between dry cleaners and launderers. Unadjusted Pearson correlation analysis of log-transformed PERC exposure indices and biomarkers of oxidative stress indicated a significant inverse association between blood PERC and leukocyte 8-OHdG ($r= -0.5812$, $P< 0.0005$), between day-1 TWA and day-2 urinary 8-OHdG ($r= -0.4826$, $P< 0.0498$). These statistically significant associations were also evident in linear models adjusted for age, body mass index, race, smoking (urinary cotinine, mg/g creatinine) and blood levels of the antioxidants vitamin E and β-carotene. The mean $\pm$ SD leukocyte 8-OHdG value in control white women was 17.8 $\pm$ 7.4 and was significantly greater than the 11.8 $\pm$ 5.9 in control black women. Smoking status was not significantly associated with any of the oxidative damage indices. Results suggest an association between PERC exposure and reduced leukocyte endogenous oxidative DNA damage.

RISK ASSESSMENT, UNCERTAINTY AND THE PRECAUTIONARY PRINCIPLE

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Numerous uncertainties, both scientific and ethical, still surround the precautionary principle. A scientific justification of precaution as a principle seems to arise first of all from the limitations of traditional toxicology underlying risk assessment. The methods of investigation of traditional toxicology seem to be inadequate to predict the reactions of organisms characterized by a high level of complexity. Traditional toxicology is essentially characterized by an analytical approach (each chemical substance is evaluated in isolation) and based on strong theoretical premises (for example a threshold of toxicity). There are some persuasive examples of how an approach based on a case by case evaluation of exposures, that excludes the overall study of interactions among environmental exposures, and relies upon strong toxicological assumptions, is deemed to be misleading: (1) two substances, aniline and norharman, taken in isolation, are not carcinogenic, but administered to rodents combine to form a powerful carcinogen; (2) in experiments conducted in heavy smokers, who received vitamins for the prevention of lung cancer, results that were opposite to those expected were observed (an excess of cancers), most likely because the intake of vitamins has a different value in subjects who already have a mutation and in subjects who do not; (3) the theory of receptors suggests that dioxin – a chemical that apparently does not cause damage to DNA – exerts its effects above a threshold, since it binds to the Ah receptor: this theory, however, is disconfirmed by epidemiologic observations in workers exposed to dioxin, who show a dose-response relationship even stronger at low levels of exposure than at high levels, with no evidence of a threshold (Steenland et al, 2001). In general, non-linear dose-response relationships in carcinogenesis need to be explained and included in risk assessment. Alternative approaches will be explored.

INTRODUCTION

APPLYING NEW BIOTECHNOLOGY TO THE STUDY OF OCCUPATIONAL CANCER

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The idea for this workshop was conceived by the NORA team for Occupational Cancer Research Methods, one of the 20 teams established to develop research agendas for the 21 NORA priority areas. During the last century, a number of human carcinogens were identified through findings of excess cancer in the workplace, and programs of toxicologic testing and regulations were developed that should provide some protection against occupational cancer in the future. Yet, occupational cancer remains an important research area for a number of reasons, including: 1) Occupation accounts for about 4% of cancer deaths - or about 24,000 deaths per year; 2) There is ongoing exposure to chemicals and occupations for which there is evidence of carcinogenicity; and 3) Fewer than 2% of chemicals in commerce have been tested for carcinogenicity. Of special concern are occupations for which there are increased risks of cancer but the specific agents are unclear, occupations involving exposure to complex mixtures, and chemicals for which both human and animal evidence for carcinogenicity exists, but is not quite strong enough to meet IARC Group 1 criteria. The challenge in occupational cancer research in the 21st century is that for many occupational exposures, traditional toxicological and epidemiological methods do not yield definitive answers. The consensus of our NORA team was that one way in which progress in occupational cancer research could be achieved is by applying newly developed technologies to the occupational issues of greatest concern. The purpose of this workshop is to bring together experts in occupational cancer with those who are working in the forefront of new technologies to identify the most important unresolved questions related to occupational cancer and the best techniques and approaches to resolve them.

Poster

CONCEPTUAL FRAMEWORK FOR THE CHEMICAL EFFECTS IN BIOLOGICAL SYSTEMS (CEBS) TOXICOGENOMICS KNOWLEDGE BASE

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Toxicogenomics studies how the genome is involved in responses to environmental stressors or toxicants. It combines genetics, genome-scale mRNA expression, cell and tissue-wide protein expression, and bioinformatics to understand the gene-environment interactions in disease. CEBS is planned as the first public toxicogenomics database combining datasets from genomics, proteomics, metabonomics and toxicology with pathway and network information relevant to environmental exposures and human disease. Standardized procedures, protocols, data formats and assessment tools will be used to assemble high quality datasets to support the design and interpretation of toxicogenomics experiments. Raw data will be available and all studies will be captured in their entirety. Dictionaries and metadata will introduce and guide interpretation of toxicogenomics datasets. CEBS will create the capability to assess the global genomic responses of biological systems to environmental stressors and to relationally link genomic data to effects data as a function of dose, time, and target cell/tissue type. CEBS will access other toxicology, biochemical pathway, genomics/proteomics resources and databases to provide information and documentation for toxicogenomics and molecular biology. It will develop relational and descriptive compendia on toxicologically important genes, groups of genes, SNPs, mutants and their functional phenotypes that are relevant to human health and environmental disease. It will enable query by compound/ structure/class, toxic/pathologic effects, gene annotation, gene groups, pathways and networks. CEBS will ultimately become a knowledge base to support hypothesis-driven research.

BIOMONITORING FOR ENVIRONMENTAL AND OCCUPATIONAL EXPOSURES: APPLICATION OF DNA MICROARRAY TECHNOLOGY

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Modulation of gene expression through exposure to environmental and occupational toxins may provide an opportunity to develop markers of biological effect. The Molecular Carcinogenesis Team (HELD/NIOSH/CDC) employs a primary normal human mammary epithelial cell (PNHMEC) model to explore this possibility. Preliminary studies have been restricted to single agent exposures (benzo[a]pyrene, oxythioquinox [morestan™, Bayer Corporation] and malathion), however, this approach is clearly applicable to complex mixture exposures (e.g., asphalt fumes, welding fumes and coke-oven emissions, among others). Gene microarrays can be used as tools for the identification of candidate biomarkers for any given exposure scenario. Our strategy is to: expose PNHMEC strains to environmental and occupational toxins (including: complex mixtures) *in vitro*; monitor changes in expression of thousands of genes as well as metabolic and signaling pathways using microarrays; and validate promising biomarkers and biomarker suites by complementary methods (including: polymerase chain reaction, TaqMan™, northern blotting). Ultimately, the long-range goal is the validation of candidate exposure biomarkers in worker populations with documented exposures. Future application of expression-based biomarkers has the potential to benefit several activities pertinent to the mission of the National Institute of Occupational Safety and Health. First, as an adjunct to epidemiological studies of occupational disease, expression biomarkers may assist in the understanding of disease pathobiology and suggest strategies for prevention and intervention. Additionally, they will be of value for genome-based risk assessment and the subsequent development of exposure standards that, once implemented by the Office of Safety and Health Administration, will protect all workers, irrespective of inherited genetic susceptibility.